AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph beginning at page 6, line 10 with the following amended paragraph:

A nucleic acid was amplified from the full-length *Fibrobacter succinogenes* 1,3-1,4-β-D-glucanase (Fsβ-D-glucanase) cDNA (Chen et al. (2001), J. Biol. Chem. 276, 17895-17901) by the PCR using the following two primers: Oligo A: 5'-CAGCCGGCGATGGCCATGGTTAGC GCA-3' and Oligo B: 5'-CTGCTAGAAGAATTCGGAGCAGGTTCGTC-3'. The amplified nucleic acid encodes a polypeptide that corresponds to a fragment from aa 24 to 272 of SEQ ID NO: 1, except that the N24 was replaced with M. The polypeptide lacks the C-terminal 78 aa of Fsβ-D-glucanase. To generate an expression vector, the amplified nucleic acid was digested with Nco I and Eco RI and then ligated into a pET26b(+) vector (~~Novagen~~ NOVAGEN, WI) that had been digested with the same enzymes. The resultant vector was confirmed by DNA sequencing. This construct, designated as pPCR-TF-glucanase, encodes a fusion protein (SEQ ID NO: 10) that has a *pel* B leading peptide sequence (KYLLPTAAAGLLLLAAQPAMA, SEQ ID NO: 11) at the N-terminus and a 19-residue segment (SEQ ID NO: 16) at the C-terminus. Once expressed in a host cell, the *pel* B leading peptide sequence was cleaved to generate a mature fusion truncated glucanase, PCR-TF-glucanase (SEQ ID NO: 9).

Please replace the paragraph beginning at page 6, line 25 with the following amended paragraph:

Another truncated Fsβ-D-glucanase (SEQ ID NO: 7), designated as "TF-glucanase," was created using PCR-based site-directed mutagenesis. This TF-glucanase lacks the just-described 19-residue segment at its C-terminus. To make a nucleic acid encoding it, a stop codon was introduced right after the codon for P248 of the just-described pPCR-TF-glucanase. A pair of complementary mutagenic primers were used. The sense strand primer has the sequence: 5'-CCTGCTCCG**TAA**TCGAGCTCC-3'. The mutagenesis was carried out in a PCR reaction mixture containing 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8), 2 mM $MgCl_2$, 0.1% Triton^R X-100, 0.1 mg/ml

nuclease-free BSA, 10-15 ng of template DNA, 0.2 mM dNTPs, 0.25 μM each of the primers, and 2.5 units of Turbo *Pfu* DNA polymerase (~~Stratagene~~ STRATAGENE, La Jolla, CA). The PCR reactions were conducted on a Hybaid TouchDown thermal cycler using the following program: 2 min at 95°C, 16 cycles of 1 min at 55°C/13 min at 68 °C/45 sec at 95 °C. The products were digested with 10 units of Dpn I at 37 °C for 1 hour (h) and subsequently transformed into *E. coli* XL-1 Blue competent cells by electroporation. The transformed cells were grown on LB agar plates containing 30 μg/ml kanamycin at 37 °C until colonies appeared on the plates. The colonies were selected randomly and cultured in 5 ml LB/ kanamycin liquid culture at 37 °C for 16 h before plasmids were isolated from the culture using a ~~QIAprep~~ QIAPREP Spin Miniprep kit (~~Qiagene~~ QIAGENE, Hilden, Germany). Mutation was confirmed by DNA sequencing. The plasmid thus obtained was named "pTF-glucanase."

Please replace the paragraph beginning at page 7, line 26 with the following amended paragraph:

The PCR products were digested with PstI and XbaI and ligated into a *Pichia* expression vector, pPICZαB (~~Invitrogen~~ INVITROGEN, CA, USA), which had been digested with the same enzymes. This vector, designated as pPICZ-TFGlu, encodes a truncated glucanase that is fused to an α factor signal sequence at its N-terminus. Once expressed in *Pichia*, the signal sequence allows the TF-glucanase to be secreted into culture medium.

Please replace the paragraph beginning at page 8, line 8 with the following amended paragraph:

The supernatants were collected by centrifugation at 8,000 x g for 15 min at 4 °C. Their volumes were reduced by 10-fold on a Pellicon Cassette concentrator (~~Millipore~~ MILLIPORE, Bedford, MA) using 10,000 M_r cut-off membranes. The concentrated supernatants were then dialyzed against 50 mM Tris-HCl buffer, pH 7.8 (buffer A) and loaded onto a Sepharose Q FF (~~Pharmacia~~ PHARMACIA, Sweden) column pre-

equilibrated with the same buffer. TF-glucanases were eluted from the column with 0 to 1 M NaCl gradient in buffer A. The homogeneity of the purified enzyme was verified by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentration was determined according to the method described in Bradford M. (1976) Anal. Biochem. 72, 248-254. Bovine serum albumin (BSA) was used as the standard.

Please replace the paragraph beginning at page 8, line 25 with the following amended paragraph:

TF glucanase was expressed in *P. pastoris* strain X-33 using the *Pichia* expression kit (~~Invitrogen~~ INVITROGEN) according to the manufacturer's instructions. Briefly, a starting culture of X-33 containing the above-described pPICZ-TFGlu was grown in 25 ml of BMGY medium (1% yeast extract, 2% peptone, 100 mM potassium phosphate, pH 6.0, 1.34% yeast nitrogen base, 4 x 10^{-5}% (w/v) biotin, and 1% glycerol) at 28 °C overnight.

Please replace the paragraph beginning at page 9, line 14 with the following amended paragraph:

1,3-1,4-β-D- glucanase enzymatic activity was also determined by measuring the rate of reducing sugar production from the hydrolysis of lichenan or barley β-glucan. Reducing sugar was measured and quantified according to the method described in Miller (1959) Anal. Chem. 31, 426-428 using glucose as the standard. Briefly, 2.7–8 mg/ml lichenan was incubated with the expressed enzyme in a 0.3 ml 50 mM sodium citrate buffer (pH 6.0) at 50 °C for 10 min. 40 μg/ml *Bacillus subtilis* lichenase (~~Megazyme~~ MEGAZYME, Ireland) was used as a reference control. The reaction was terminated by the addition of salicylic acid solution. Data were analyzed using the computer program ENZFITTER (BIOSOFT, USA) and Enzyme Kinetics (SigmaPlot 2000, SPSS Inc.) Data were analyzed using the computer program ENZFITTER (BIOSOFT, USA) and Enzyme Kinetics (~~SigmaPlot~~ SIGMAPLOT 2000, SPSS Inc.) One unit (U) of enzyme activity was defined as the amount of enzyme required to release one μmol of reducing sugar (e.g., glucose equivalent) per minute. The activity was expressed in μmoles of glucose per minute per milligram of protein.

Please replace the paragraph beginning at page 10, line 3 with the following amended paragraph:

SDS-PAGE was carried out and showed that that more than 90% of the TF-glucanase existed as two dominant glycosylated forms. Homogeneous glycosylated TF-glucanase (> 96% purity) was obtained using Q-anion ion exchange column chromatography. The glycoside moiety of the glycosylated-TF-glucanase could be removed after digestion with glycosidases using the Enzymatic Deglycosylation Kit (~~Bio-Rad~~ BIO-RAD). The resultant single band of the *P. pastoris*-expressed TF-glucanase had mobility on SDS gel similar to that of the *E. coli.*-expressed TF-glucanase

Please replace the paragraph beginning at page 10, line 15 with the following amended paragraph:

Electrospray ionization-tandem mass spectrometry was carried out to determined the molecular mass of the above-described glucanases. 10 nmole of protein sample was analyzed on a LCQ (~~Finnigan~~ FINNIGAN LCQ, USA) ion trap mass spectrometer operated in full-scan MS mode (400.00-2000.00). The molecular masses of Fsβ-glucanases, the two glycosylated forms of TF-glucanases, the deglycosylated TF-glucanase were found to be 37,669, 31,850, 29,983, and 27,957 Da, respectively. These results indicate that the two glycosylated glucanases consisted of 24 and 12 glycosides, respectively. The *E. coli*-expressed PCR-TF-glucanase and TF-glucanase were also examined and found to have molecular weights of 29,722 and 27,744 Da, respectively.

Please replace the paragraph beginning at page 10, line 24 with the following amended paragraph:

The enzymatic activities of Fsβ-glucanase, TF-glucanase, PCR-TF-glucanase, PCR-TF-W203F, Glycosylated TF-Glucanase, and Lichanase (~~Megazyme~~ MEGAZYME, Ireland) were determined by the lichenan-hydrolysis assay described above. The results are shown in Table 1 below.

Please replace the paragraph beginning at page 14, line 3 with the following amended paragraph:

A Bacillus lichenase (~~Megazyme~~ MEGAZYME) was also examined and was found to be more heat-sensitive than TF-glucanase. After being heated at 90 °C for 10 minute, its activity was hardly restored (<10 %) after recovering for 3 minutes to 2 hours, and was lost completely after recovering for 4 to 6 hours.

Please replace the paragraph beginning at page 15, line 6 with the following amended paragraph:

The structural integrity of native, heat-denatured, and denatured-recovered wild type and PCR-TF-glucanases were analyzed using fluorescence spectrometry. Enzyme samples (0.03 mg/ml in 50 mM sodium phosphate, pH 7.0) were heated at 90°C and recovered at 25 °C for 0, 3, and 10 min, respectively. Their spectra from 305 nm to 430 nm of fluorescence emission excited by 295 nm light were recorded on an AMICO-Bowman Series 2 spectrofluorimeter (~~Spectronic Instruments~~ SPECTRONIC INSTRUMENTS, Inc., NY) at 25°C with a 1 x 1-cm cuvette. A 4-nm slit was used for the recordation.